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Attorney Docket No. 15966-729 (Cura-229)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT: Taupier, Jr., et al
SERIAL NUMBER: 09/813,432 EXAMINER: Lazar Wesley
FILING DATE: 03/20/01 ART UNIT: 1646
FOR: NOVEL POLYPEPTIDES AND NUCLEIC ACIDS ENCODING SAME

Commissioner for Patents
Washington, D.C. 20231

DECLARATION UNDER 37 C.F.R. § 1.132

I, Valerie Gerlach, hereby declare and state as follows:

1. I am employed by CuraGen, Inc., the assignee of this application. My title is senior research scientist. I received a Ph.D. in Cellular and Molecular Biology in 1996 from the University of Wisconsin-Madison. I was a post-doctoral fellow in the laboratory of Dr. Errol Friedberg at the University of Texas Southwestern Medical Center in Dallas, Texas from 1996 to 2000.

2. I have read, and am familiar with, the contents of the United States patent application entitled "Novel Polypeptides and Nucleic Acids Encoding Same", serial number 09/813,432 which was filed March 20, 2001. I understand that the pending claims are directed to a polypeptide NOV 11, SEQ ID NO: 22, also known as CG54656-05.

3. I am aware that the Examiner has issued an Office Action. In particular, I understand that the Examiner has rejected the pending claims under 35 U.S.C. §§ 101 and 112, contending that the pending claims are not supported by either a specific and substantial asserted utility or a well-established utility.

4. I make this declaration to rebut the Examiner's assertion, with which I do not agree. It is my opinion and belief that the claimed compositions have a specific and substantial utility for at least the following reasons.

5. I have performed, or have had performed under my supervision, studies evaluating the quantitative expression and sequence homology of the nucleic acid of SEQ ID NO: 21 encoding the polypeptide of SEQ ID NO: 22 (also known as CG54656-05) in tissue culture cells and in isolated normal and pathological human tissue. The methods used to perform these studies are described in the Appendix attached hereto.

6. In a first study, results provided in Table 1 in the Appendix using specific probe/primer set (Ag545, which are shown in Table A), show that expression of the NOV11, CG54656-05 gene is upregulated in fetal brain (CT=28), with low to moderate expression seen in whole brain, pituitary, and amygdala samples. Thus, gene or proteins levels of expression are useful as a marker of brain tissue. Furthermore, therapeutic modulation of the expression or function of this gene is useful in the treatment of neurological disorders, such as Alzheimer's disease, Parkinson's disease, schizophrenia, multiple sclerosis, stroke and epilepsy.

7. In a second study, results using Ag517/545 probe/primer sets, shown in Table 2 in the Appendix, show increased expression of the CG54656-05 gene in a cluster of samples derived from breast cancer (CTs=31-33). Thus, gene or proteins levels of expression are useful to differentiate between these samples and other samples on this panel and as a marker to detect the presence of breast cancer. Furthermore, gene, protein, antibodies or small molecule therapeutics targeting this gene or its protein product are effective in the treatment of breast cancer.

9. The results of these studies, in my opinion and belief, demonstrate that the polypeptide can be used in therapeutic and diagnostic applications in neurological disorders and breast cancer. Thus, I believe that the Examiner should withdraw the rejection and allow the pending claims.

10. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001, Title 18, United

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States Code, and that willful false statements may jeopardize the validity of this application and any patent issuing therefrom.

Valerie Gerlach

Valerie Gerlach

Signed at Branford CT
this 21ST day of April

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Appendix

Quantitativ expression analysis f CG51400-02 in various cells and tissues

The quantitative expression of various clones was assessed using microtiter plates containing RNA samples from a variety of normal and pathology-derived cells, cell lines and tissues using real time quantitative PCR (RTQ PCR). RTQ PCR was performed on an Applied Biosystems ABI PRISM® 7700 or an ABI PRISM® 7900 HT Sequence Detection System. Various collections of samples are assembled on the plates, and referred to as Panel 1 (containing normal tissues and cancer cell lines), and Panel 2 (containing samples derived from tissues from normal and cancer sources).

RNA integrity from all samples is controlled for quality by visual assessment of agarose gel electropherograms using 28S and 18S ribosomal RNA staining intensity ratio as a guide (2:1 to 2.5:1 28s:18s) and the absence of low molecular weight RNAs that would be indicative of degradation products. Samples are controlled against genomic DNA contamination by RTQ PCR reactions run in the absence of reverse transcriptase using probe and primer sets designed to amplify across the span of a single exon.

First, the RNA samples were normalized to reference nucleic acids such as constitutively expressed genes (for example, β-actin and GAPDH). Normalized RNA (5 ul) was converted to cDNA and analyzed by RTQ-PCR using One Step RT-PCR Master Mix Reagents (Applied Biosystems; Catalog No. 4309169) and gene-specific primers according to the manufacturer's instructions.

In other cases, non-normalized RNA samples were converted to single strand cDNA (sscDNA) using Superscript II (Invitrogen Corporation; Catalog No. 18064-147) and random hexamers according to the manufacturer's instructions. Reactions containing up to 10 μg of total RNA were performed in a volume of 20 μl and incubated for 60 minutes at 42°C. This reaction can be scaled up to 50 μg of total RNA in a final volume of 100 μl. sscDNA samples are then normalized to reference nucleic acids as described previously, using 1X TaqMan® Universal Master mix (Applied Biosystems; catalog No. 4324020), following the manufacturer's instructions.

Probes and primers were designed for each assay according to Applied Biosystems Primer Express Software package (version I for Apple Computer's Macintosh Power PC) or a similar

algorithm using the target sequence as input. Default settings were used for reaction conditions and the following parameters were set before selecting primers: primer concentration = 250 nM, primer melting temperature (Tm) range = 58°-60°C, primer optimal Tm = 59°C, maximum primer difference = 2°C, probe does not have 5'G, probe Tm must be 10°C greater than primer Tm, amplicon size 75bp to 100bp. The probes and primers selected (see below) were synthesized by Synthegen (Houston, TX, USA). Probes were double purified by HPLC to remove uncoupled dye and evaluated by mass spectroscopy to verify coupling of reporter and quencher dyes to the 5' and 3' ends of the probe, respectively. Their final concentrations were: forward and reverse primers, 900nM each, and probe, 200nM.

PCR conditions: When working with RNA samples, normalized RNA from each tissue and each cell line was spotted in each well of either a 96 well or a 384-well PCR plate (Applied Biosystems). PCR cocktails included either a single gene specific probe and primers set, or two multiplexed probe and primers sets (a set specific for the target clone and another gene-specific set multiplexed with the target probe). PCR reactions were set up using TaqMan® One-Step RT-PCR Master Mix (Applied Biosystems, Catalog No. 4313803) following manufacturer's instructions. Reverse transcription was performed at 48°C for 30 minutes followed by amplification/PCR cycles as follows: 95°C 10 min, then 40 cycles of 95°C for 15 seconds, 60°C for 1 minute. Results were recorded as CT values (cycle at which a given sample crosses a threshold level of fluorescence) using a log scale, with the difference in RNA concentration between a given sample and the sample with the lowest CT value being represented as 2 to the power of delta CT. The percent relative expression is then obtained by taking the reciprocal of this RNA difference and multiplying by 100.

When working with sscDNA samples, normalized sscDNA was used as described previously for RNA samples. PCR reactions containing one or two sets of probe and primers were set up as described previously, using 1X TaqMan® Universal Master mix (Applied Biosystems; catalog No. 4324020), following the manufacturer's instructions. PCR amplification was performed as follows: 95°C 10 min, then 40 cycles of 95°C for 15 seconds, 60°C for 1 minute. Results were analyzed and processed as described previously.

Panel 1.2

The plates for Panel 1.2 include 2 control wells (genomic DNA control and chemistry control) and 94 wells containing cDNA from various samples. The samples in these panels are broken into 2 classes: samples derived from cultured cell lines and samples derived from primary normal tissues. The cell lines are derived from cancers of the following types: lung cancer, breast cancer, melanoma, colon cancer, prostate cancer, CNS cancer, squamous cell carcinoma, ovarian cancer, liver cancer, renal cancer, gastric cancer and pancreatic cancer. Cell lines used in these panels are widely available through the American Type Culture Collection (ATCC), a repository for cultured cell lines, and were cultured using the conditions recommended by the ATCC. The normal tissues found on these panels are comprised of samples derived from all major organ systems from single adult individuals or fetuses. These samples are derived from the following organs: adult skeletal muscle, fetal skeletal muscle, adult heart, fetal heart, adult kidney, fetal kidney, adult liver, fetal liver, adult lung, fetal lung, various regions of the brain, the spleen, bone marrow, lymph node, pancreas, salivary gland, pituitary gland, adrenal gland, spinal cord, thymus, stomach, small intestine, colon, bladder, trachea, breast, ovary, uterus, placenta, prostate, testis and adipose.

In the results for Panel 1.2, the following abbreviations are used:
ca. = carcinoma,
* = established from metastasis,
met = metastasis,
s cell var = small cell variant,
non-s = non-sm = non-small,
squam = squamous,
pl. eff = pl effusion = pleural effusion,
glio = glioma,
astro = astrocytoma, and
neuro = neuroblastoma.

Panel 2D

The plates for Panel 2D generally include 2 control wells and 94 test samples composed of RNA or cDNA isolated from human tissue procured by surgeons working in close cooperation with the National Cancer Institute's Cooperative Human Tissue Network (CHTN) or the National Disease Research Initiative (NDRI) or from Ardais or Clinomics). The tissues are derived from human malignancies and in cases where indicated many malignant tissues have "matched margins" obtained from noncancerous tissue just adjacent to the tumor. These are termed normal adjacent tissues and are denoted "NAT" in the results below. The tumor tissue and the "matched margins" are evaluated by two independent pathologists (the surgical pathologists and again by a pathologist at NDRI/ CHTN/Ardais/Clinomics). Unmatched RNA samples from tissues without malignancy (normal tissues) were also obtained from Ardais or Clinomics. This analysis

provides a gross histopathological assessment of tumor differentiation grade. Moreover, most samples include the original surgical pathology report that provides information regarding the clinical stage of the patient. These matched margins are taken from the tissue surrounding (i.e. immediately proximal) to the zone of surgery (designated "NAT", for normal adjacent tissue, in Table RR). In addition, RNA and cDNA samples were obtained from various human tissues derived from autopsies performed on elderly people or sudden death victims (accidents, etc.). These tissues were ascertained to be free of disease and were purchased from various commercial sources such as Clontech (Palo Alto, CA), Research Genetics, and Invitrogen.

Expression of gene CG54656-05 was assessed using the primer-probe sets Ag517, and Ag545, described in Tables AA, and AB. Results of the RTQ-PCR runs are shown in Tables AC and AD.

Table AA Probe Name Ag517

Primers	Sequences	Length	Start Position	SEQ ID No
Forward	5'-cgcacccggaaagtcatt-3'	18	431	
Probe	TET-5'-taagtgtttacatcacctgcttcctgaccagc-3'-TAMRA	32	450	
Reverse	5'-tgttgggccaccagtaatagg-3'	21	486	

Table AB Probe Name Ag545

Primers	Sequences	Length	Start Position	SEQ ID No
Forward	5'-cgtctccgtggctactcca-3'	19	650	
Probe	TET-5'-accaccgccatcttgttcaccattacct-3'-TAMRA	28	677	
Reverse	5'-gcccaaagtgtggcaaagat-3'	20	707	

Table AC Panel 1.2

Column A - Rel. Exp.(%) Ag545, Run 112162286			
Tissue Name	A	Tissue Name	A

Endothelial cells	0.0	Renal ca. 786-0	0.0
Heart (Fetal)	0.0	Renal ca. A498	0.6
Pancreas	0.0	Renal ca. RXF 393	0.0
Pancreatic ca. CAPAN 2	0.0	Renal ca. ACHN	0.1
Adrenal Gland	0.0	Renal ca. UO-31	0.3
Thyroid	0.0	Renal ca. TK-10	0.3
Salivary gland	0.4	Liver	0.0
Pituitary gland	26.4	Liver (fetal)	0.0
Brain (fetal)	**100.0**	Liver ca. (hepatoblast) HepG2	0.0
Brain (whole)	2.6	Lung	0.0
Brain (amygdala)	1.5	Lung (fetal)	0.0
Brain (cerebellum)	0.0	Lung ca. (small cell) LX-1	0.0
Brain (hippocampus)	2.0	Lung ca. (small cell) NCI-H69	17.4
Brain (thalamus)	7.2	Lung ca. (s.cell var.) SHP-77	0.1
Cerebral Cortex	0.5	Lung ca. (large cell)NCI-H460	0.5
Spinal cord	0.8	Lung ca. (non-sm. cell) A549	3.4
glio/astro U87-MG	0.0	Lung ca. (non-s.cell) NCI-H23	0.0
glio/astro U-118-MG	0.0	Lung ca. (non-s.cell) HOP-62	0.2
astrocytoma SW1783	0.0	Lung ca. (non-s.cl) NCI-H522	0.0
neuro*; met SK-N-AS	0.1	Lung ca. (squam.) SW 900	0.0
astrocytoma SF-539	0.0	Lung ca. (squam.) NCI-H596	3.1
astrocytoma SNB-75	0.0	Mammary gland	0.0
glioma SNB-19	0.1	Breast ca.* (pl.ef) MCF-7	4.6
glioma U251	0.0	Breast ca.* (pl.ef) MDA-MB-231	0.0
glioma SF-295	0.0	Breast ca.* (pl. ef) T47D	5.1
Heart	0.0	Breast ca. BT-549	0.1
Skeletal Muscle	0.2	Breast ca. MDA-N	1.0
Bone marrow	4.5	Ovary	0.0
Thymus	0.0	Ovarian ca. OVCAR-3	0.1
Spleen	0.0	Ovarian ca. OVCAR-4	0.0
Lymph node	0.0	Ovarian ca. OVCAR-5	9.0
Colorectal Tissue	0.2	Ovarian ca. OVCAR-8	0.1
Stomach	0.0	Ovarian ca. IGROV-1	0.6
Small intestine	0.0	Ovarian ca. (ascites) SK-OV-3	0.1
Colon ca. SW480	0.0	Uterus	0.0
Colon ca.* SW620 (SW480 met)	0.0	Placenta	0.0
Colon ca. HT29	0.2	Prostate	0.0

Colon ca. HCT-116	0.0	Prostate ca.* (bone met) PC-3	0.1
Colon ca. CaCo-2	0.0	Testis	5.0
Colon ca. Tissue (ODO3866)	2.1	Melanoma Hs688(A).T	0.3
Colon ca. HCC-2998	0.0	Melanoma* (met) Hs688(B).T	4.7
Gastric ca.* (liver met) NCI-N87	0.1	Melanoma UACC-62	0.1
Bladder	4.2	Melanoma M14	2.1
Trachea	0.0	Melanoma LOX IMVI	0.8
Kidney	0.0	Melanoma* (met) SK-MEL-5	0.7
Kidney (fetal)	0.0		

Table AD Panel 2D

Column A - Rel. Exp.(%) Ag517, Run 145719283 Column B - Rel. Exp.(%) Ag545, Run 145553420 Column C - Rel. Exp.(%) Ag545, Run 145728111							
Tissue Name	**A**	**B**	**C**	**Tissue Name**	**A**	**B**	**C**
Normal Colon	0.7	5.0	1.5	Kidney Margin 8120608	0.0	0.0	0.0
CC Well to Mod Diff (ODO3866)	0.0	0.0	2.5	Kidney Cancer 8120613	0.0	1.6	0.0
CC Margin (ODO3866)	1.3	0.4	1.0	Kidney Margin 8120614	0.5	0.0	1.6
CC Gr.2 rectosigmoid (ODO3868)	0.0	0.0	1.9	Kidney Cancer 9010320	0.0	0.0	0.0
CC Margin (ODO3868)	0.0	0.0	0.0	Kidney Margin 9010321	0.0	0.0	0.0
CC Mod Diff (ODO3920)	0.0	3.8	1.9	Normal Uterus	0.0	0.0	0.0
CC Margin (ODO3920)	0.0	0.4	0.0	Uterus Cancer 064011	0.9	0.0	0.0
CC Gr.2 ascend colon (ODO3921)	0.0	0.0	3.1	Normal Thyroid	0.0	0.0	0.0
CC Margin (ODO3921)	0.0	0.9	2.2	Thyroid Cancer 064010	0.0	0.0	1.0
CC from Partial Hepatectomy (ODO4309) Mets	0.0	0.0	0.0	Thyroid Cancer A302152	0.0	1.9	0.0
Liver Margin (ODO4309)	1.4	0.0	1.0	Thyroid Margin A302153	0.0	0.0	0.0
Colon mets to lung (OD04451-01)	0.0	0.5	0.0	Normal Breast	1.0	0.9	0.7
Lung Margin (OD04451-02)	0.0	0.0	0.0	Breast Cancer (OD04566)	17.9	18.2	38.7
Normal Prostate 6546-1	0.0	0.0	2.3	Breast Cancer (OD04590-01)	**100.0**	**100.0**	85.9
Prostate Cancer (OD04410)	0.0	0.0	0.0	Breast Cancer Mets (OD04590-03)	51.4	48.6	46.7
Prostate Margin (OD04410)	0.0	0.0	0.0	Breast Cancer Metastasis (OD04655-05)	76.8	84.1	**100.0**
Prostate Cancer (OD04720-01)	0.0	0.0	0.0	Breast Cancer 064006	0.0	0.0	0.0
Prostate Margin (OD04720-02)	0.0	0.0	0.0	Breast Cancer 1024	0.0	0.0	0.0
Normal Lung 061010	0.0	2.0	0.0	Breast Cancer 9100266	12.2	6.5	2.6

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Lung Met to Muscle (ODO4286)	0.0	0.0	1.9	Breast Margin 9100265	1.0	0.0	1.8
Muscle Margin (ODO4286)	0.0	0.0	0.0	Breast Cancer A209073	2.0	1.9	0.7
Lung Malignant Cancer (OD03126)	0.0	0.0	1.8	Breast Margin A209073	3.9	0.6	5.6
Lung Margin (OD03126)	0.0	0.0	0.7	Normal Liver	0.0	0.0	0.0
Lung Cancer (OD04404)	0.0	0.9	0.0	Liver Cancer 064003	0.0	1.7	0.0
Lung Margin (OD04404)	0.0	0.0	0.0	Liver Cancer 1025	0.0	0.0	0.0
Lung Cancer (OD04565)	0.0	0.0	0.0	Liver Cancer 1026	0.0	0.0	0.0
Lung Margin (OD04565)	0.0	0.0	0.0	Liver Cancer 6004-T	0.0	0.0	0.0
Lung Cancer (OD04237-01)	0.0	0.0	0.0	Liver Tissue 6004-N	0.0	1.7	1.0
Lung Margin (OD04237-02)	0.0	1.8	0.0	Liver Cancer 6005-T	0.0	0.0	0.0
Ocular Mel Met to Liver (ODO4310)	0.0	0.0	0.0	Liver Tissue 6005-N	1.8	0.0	0.0
Liver Margin (ODO4310)	0.0	0.0	3.5	Normal Bladder	1.0	0.0	1.9
Melanoma Mets to Lung (OD04321)	0.0	0.0	1.4	Bladder Cancer 1023	0.0	2.7	0.9
Lung Margin (OD04321)	0.0	1.7	0.0	Bladder Cancer A302173	2.7	1.1	2.0
Normal Kidney	0.0	0.0	0.0	Bladder Cancer (OD04718-01)	0.0	0.0	0.0
Kidney Ca, Nuclear grade 2 (OD04338)	1.6	0.0	0.0	Bladder Normal Adjacent (OD04718-03)	2.0	0.0	3.0
Kidney Margin (OD04338)	0.0	0.0	0.0	Normal Ovary	0.6	0.0	0.0
Kidney Ca Nuclear grade 1/2 (OD04339)	0.0	0.0	0.0	Ovarian Cancer 064008	0.0	0.5	0.0
Kidney Margin (OD04339)	0.0	0.0	1.7	Ovarian Cancer (OD04768-07)	0.0	1.7	0.0
Kidney Ca, Clear cell type (OD04340)	0.0	0.0	0.0	Ovary Margin (OD04768-08)	0.0	0.0	2.1
Kidney Margin (OD04340)	0.0	0.0	0.0	Normal Stomach	0.0	2.1	0.0
Kidney Ca, Nuclear grade 3 (OD04348)	0.0	0.0	1.9	Gastric Cancer 9060358	0.0	1.6	1.6
Kidney Margin (OD04348)	2.2	0.0	0.0	Stomach Margin 9060359	0.0	0.0	0.0
Kidney Cancer (OD04622-01)	0.0	1.0	0.0	Gastric Cancer 9060395	0.0	0.0	0.0
Kidney Margin (OD04622-03)	1.8	0.0	0.8	Stomach Margin 9060394	0.0	0.0	0.0
Kidney Cancer (OD04450-01)	0.0	0.0	1.0	Gastric Cancer 9060397	1.0	2.0	1.1
Kidney Margin (OD04450-03)	0.0	0.0	0.0	Stomach Margin 9060396	0.0	0.0	0.0
Kidney Cancer 8120607	0.0	2.0	0.0	Gastric Cancer 064005	0.0	1.8	0.8

Panel 1.2 Summary: Ag545 Highest expression is detected in fetal brain (CT=28), with low to moderate expression seen in whole brain, pituitary, and amygdala samples. Thus, gene or proteins levels of expression are useful as a marker of brain tissue. Furthermore, therapeutic modulation of the expression or function of this gene is useful in the treatment of neurological disorders, such as Alzheimer's disease, Parkinson's disease, schizophrenia, multiple sclerosis, stroke and epilepsy. In addition, this gene shows low but significant levels of expression in a cluster of breast cancer cell lines, consistent with expression in Panel 2D.

Panel 2D Summary: Ag517/Ag545 Expression is seen only in a cluster of samples derived from breast cancer (CTs=31-33). Thus, gene or proteins levels of expression are are useful to differentiate between these samples and other samples on this panel and as a marker to detect the presence of breast cancer. Furthermore, gene, protein, antibodies or small molecule therapeutics targeting this gene or its protein product are effective in the treatment of breast cancer.

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